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FACTORS THAT MODIFY THE BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN THE ENDOPLASMIC RETICULUM

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ABSTRACT. The effect of different factors upon fatty acid desaturation in the endoplasmic reticulum was studied and compared. The factors that modify the fatty acid desaturation are: competition among different fatty acids, competition between desaturation and transacylation of lipids, ATP, fasting, carbohydrates and proteins in the diet, diabetes and insulin. Besides, it was also found that the desaturation of fatty acids follows a circadian rhythm.

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The liver has a very important function in the total lipid metabolism of the organism. The liver not only receives the fatty acids from the intestines or the adipose tissue to either oxidize or transform them by elongation or desaturation into other fatty acids, but it can synthetize de novo mono-unsaturated (monoethylenic) and saturated acids. These synthetized or transformed fatty acids may be transported intact by the blood stream before the formation to triglycerides, phospholipids and cholesterol esters bound to proteins. The blood lipoproteins α, β, and pre-β are produced in the liver and allow, among other things, the distribution of the fatty acids in the organism.

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**Numbers in the margin indicate pagination in the original foreign text.

In 1965, Nervi and Brenner [1], administering the essential fatty acids linoleic or arachidonic to rats kept in a diet deficient in essential fatty acids, found that the liver rapidly synthetized arachidonic acid from linoleic acid. Such a synthesis is apparently of little importance in other organs, such as the heart. It was suggested that arachidonic acid, a fatty acid fundamental for the constitution of phospholipids, cellular membranes, prostaglandins, etc., arises either from foods of animal origin or from synthesis from linoleic acid, primarily by the liver. This situation probably occurs with other polyethylenic acids as well. As a consequence, it became important to study the mechanism of synthesis of polyunsaturated (polyethylenic) fatty acids in the liver, and their regulation.

The unsaturated fatty acids are synthetized via two reactions. In the first, double bonds are introduced; in the other reaction, elongation takes place by consecutive addition of two carbons. Both reactions take place in the endoplasmic reticulum. The desaturation reaction takes place with the intervention of the microsomal electron carrier system [2]. It requires NADH or NADPH and O_2. The fatty acid is previously converted by reticular enzymes to the CoA derivative. The unsaturated fatty acid is then incorporated into lipids or elongated and desaturated again to form higher fatty acids. As an example, we show the desaturation of linoleic acid to γ-linoleic [3], which is the first step for the synthesis of other acids in the linoleic family. (See Figure 1.)

Apparently there are different desaturases, but the information obtained so far indicates that the linoleic acid desaturase (6-desaturase) is the same enzyme that desaturates oleic to octadecadienoic and the α-linoleic to octadecatetraenoic [1].

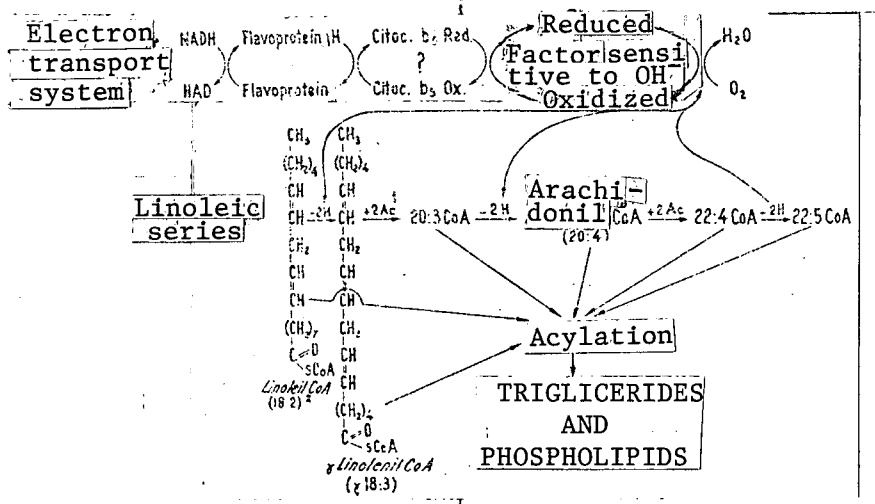

Figure 1. Scheme of the biosynthesis of fatty acids of the linoleic series in the endoplasmic reticulum

[1]AC = acetate

[2]Fatty acids are identified by two numbers, the first one indicates the number of carbon atoms and the second the number of double bonds. Example: 18:2 = linoleic

However, it is different from the enzyme that changes stearic into oleic called "9 desaturase". They are named 6 desaturase and 9 desaturase due to the position in which the new double bond is formed. The 6 desaturase introduces it between carbon atoms 6 - 7, and the 9 desaturase — between 9 - 10. /144

Experimental Conditions

The reaction of desaturation of linoleic acid and other non-saturated fatty acids was studied in microsomes obtained from rat liver by differential centrifugation at 100,000 × g for one hour [4]. The microsomes (1 to 5 mg of protein) were incubated at 35° in phosphate buffer, pH 7.0, under different experimental conditions, either with acyl CoA or with free acid. In the

latter case ATP (4 micromoles), $MgCl_2$ (1.5 micromoles) and CoA (0.2 micromoles) were added to form the acyl CoA derivative. In all cases NADH (2.5 micromoles) was added and the mixture incubated in air. The total volume was 3 ml. The incubation time was generally extended to 20 minutes in order to bring the reaction to completion. As substrates, carboxyl ^{14}C labeled acids were used, and the amount of acid transformed was measured by gas-liquid radiochromatography in a Pye apparatus equipped with a proportional detector, using columns of diethylene glycol succinate (10%) on Chromosorb W (80 - 100 mesh). The radioactive acids employed (linoleic 1-^{14}C, α-linoleic 1-^{14}C, oleic 1-^{14}C, stearic 1-^{14}C and palmitic 1-^{14}C) were provided by the Radiochemical Center, Amersham, England, and were over 98% pure. The non-radioactive acids were provided by the Hormel Institute, Austin, Minnesota, and were better than 99% pure.

Results

In this way it was possible to demonstrate that the desaturation of fatty acids in the hepatic endoplasmic reticulum can be modified by different factors. These factors are:

1) Competition among different unsaturated acids. It has been possible to show that different cis or trans unsaturated acids of the same or different series compete in the unsaturation of linoleic acid to γ-linoleic [3, 4, 5, 6] (see Figure 2).

2) Under normal cellular in vivo conditions, the relation enzyme/substrate is generally displaced towards an excess of enzyme. In other words, the conditions are different from those usually employed in vitro to obtain kinetic data. By utilizing the earlier conditions with hepatic microsomes, we were able to show that the incorporation of linoleic acid into lipids

(phospholipids and triglycerides) competes with the unsaturation reaction. This phenomenon can modify the yield of the desaturation [7]. This is shown in Table 1. The preincubation of the acid with microsomes under conditions in which desaturation occurs at below optimal conditions results in incorporation into lipids and a diminished desaturation because of a lowered amount of convertible substrate. On the other hand, the preincubation of the microsomes with unlabeled acid under non-optimal conditions followed by incubation in a desaturating medium with the labeled fatty acid increases the desaturation of the latter by diminishing its incorporation into lipids.

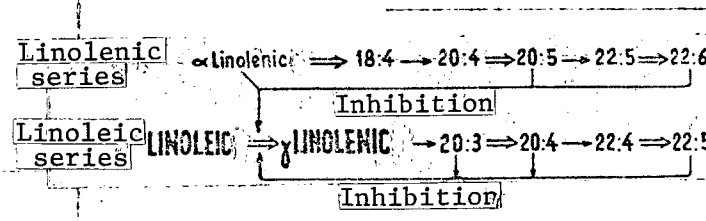

Figure 2. Fatty acids from the linoleic and linolenic series that inhibited the desaturation of linoleic to γ-linolenic

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3) Isolated microsomes, when preincubated under nitrogen with ATP, increase their yield of desaturation of linoleic, α linolenic and oleic, but do not modify the -9 desaturation (Tables 1 and 2). This effect seems to be due to a modification of the microsomes and is not due to GTP, CTP, ADP, or AMP [2].

4) The composition of the food modifies the yield of desaturation of unsaturated acids in the microsomal in vitro system. When measuring the capacity of desaturation of linoleic acids to γ linolenic in microsomes from rats kept fasted for increasing periods of time, Brenner et al. [8] and I. N. T. de Gomez Dumm et al. [9] showed that fasting diminishes the desaturation of both the 6 and 9 desaturases (see Figure 3).

TABLE 1. EFFECT OF THE ACYLATION OF THE LIPIDS IN THE DESATURATION OF LINOLEIC TO γ LINOLENIC

5 mg of microsomal protein were preincubated 15 min at 35° under N_2 with cofactors specified previously at pH 7.0. Then they were incubated at 35° for 20 minutes under O_2 with the previous addition of 2.5 µmoles NADH and 10 n moles of linoleic 1-^{14}C when specified. Desaturation was measured in an aliquot by TLC using Cl_3CH: CH_3OH: H_2O (65:25:4 V/V/V) and n hexane; ethyl ether; acetic acid (80:20:1 V/V/V) and then counting radioactivity

Added cofactors: Preincubation	Incubation	Radioactivity distribution, % 1 PC	PE	AG	DG	TG	Variation in % of desaturation
18:2—1 ^{14}C + ATP + Mg++ + CoA	NADH	60,1	8,4	12,8	7,2	8,0	—31,9
ATP + Mg++ + CoA	NADH + 18:2—1—^{14}C	63,1	5,0	7,1	11,6	5,0	+15,4
18:2 (40 nmoles) + ATP + Mg++ + CoA	NADH + 18:2—1—^{14}C	18,0	4,7	38,4	13,4	8,8	+28,6
	NADH + 18:2—1—^{14}C + ATP + Mg++ + CoA 18:2 (40 nmoles)	11,2	2,2	46,9	20,5	11,2	—21,6
	NADH + 18:2—1—^{14}C + ATP + Mg++ + CoA	67,4	7,2	1,9	10,2	8,7	0 [2]

[1] PC choline glycerophosphatides; PE ethanolamine glycerophosphatides; AG fatty acids; DG diglycerides; TG triglycerides. Minor components complete the %.

[2] Corresponding to 15.7% desaturation.

* Commas represent decimal points.

TABLE 2. EFFECT OF PREINCUBATION OF MICROSOMES WITH ATP ON THE DESATURATION OF PALMITIC, STEARIC, OLEIC, LINOLEIC AND α LINOLENIC

Microsomes were preincubated 15 min in N_2 at 35° with ATP (2.5 µmoles). Subsequent incubation was carried out after adding 15 µmoles of $MgCl_2$; 0.2 µmoles CoA; 5 nmoles of 1-^{14}C acid. 5 mg of microsomal proteins were used in a total final volume of 3 ml

Acid	Conversion	
	Preincubation with ATP	Without preincubation
Linoleic	26.5	20.0
Oleic	4.0	2.8
α linolenic	56.4	44.4
Stearic	5.8	7.3
Palmitic	7.1	9.7

When the animals were re-fed after 96 hours of fasting, a different response was founded according to the composition of the diet. When glucose was given, it produced an increase in the saturation of linoleic to γ-linolenic to normal values that were low again after 4 hours. On the other hand, casein causes an increase in the desaturation -6 that reaches higher levels and lasted for longer periods of time [10]. The reasons for such behavior are not easy to explain due to the enzymatic complexity of the microsomes.

6) Hormones modify the desaturating capacity of the microsomes. The desaturations 6 and 9 are diminished in diabetes, and normal levels are achieved by insulin treatment *in vivo* but not in the *in vitro* system [11, 12]. This could be related to the effects of fasting and carbohydrate and protein diets.

6) Existence of a circadian cycle. By utilizing mice, we have recently been able to show the existence of a diurnal variation in the desaturation of linoleic and stearic acids (see Figure 4). This variation has to be related with the cyclic consumption of foods and the corresponding hormone balance.

In addition to the liver, which is the most active organ in the synthesis of polyunsaturated fatty acids from its precursors, synthesis of these acids occurs in other organs. This has been shown for testicles [8, 13] and the suprarenal glands [1]. The partial dependence of the testicle with respect to

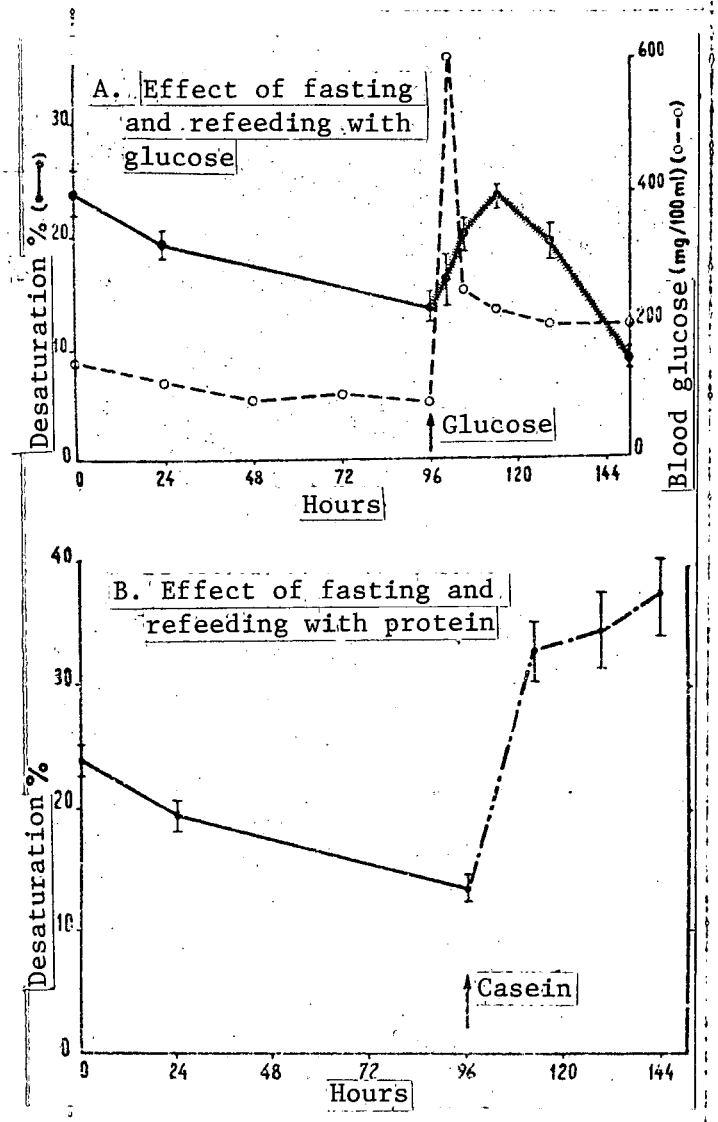

Figure 3. Effect of fasting, carbohydrates and proteins in the desaturation of linoleic acid to α linolenic. Desaturation % ± E.S. Determined with 5 mg of microsomal proteins from rat liver under the conditions described in the text for 20 minutes under air. Five animals per group. Fasted animals were refed with 50% glucose solution or 20% casein.

the liver in the synthesis of polyunsaturated acids is very important and necessary, due to the fact that testicle maturation and functioning do not parallel the hepatic rhythms.

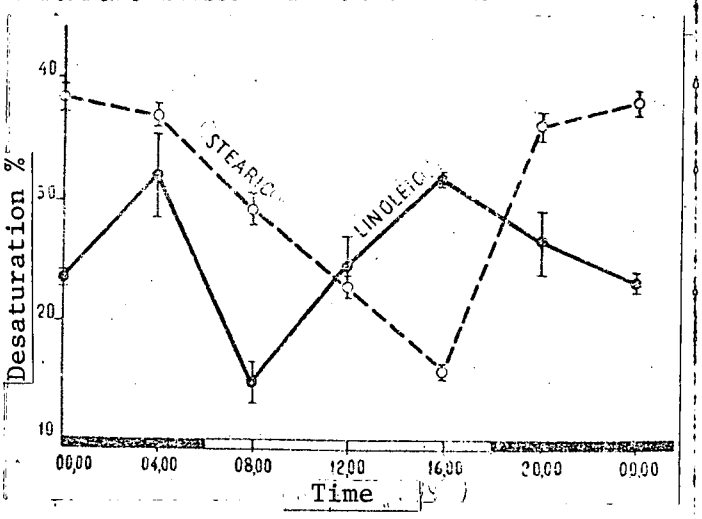

Figure 4. Circadian rhythm in the desaturation of linoleic and stearic acids. Desaturation measured in liver microsomes from female mice (C3H-s) 6 weeks of age obtained from the Instituto de Embriologia, Biologia e Histologia de la Facultad de Ciencias Medicas de la Plata. Five mg of microsomal protein were incubated 20 min at 35° with 10 nmoles of acids under the conditions described. Each point represents the mean of six animals. Desaturation ± S.E.

In conclusion, the synthesis of polyunsaturated fatty acids in the liver is regulated by fast acting factors, such as competitive reactions, or more slow ones such as food consumption and hormones. All of these relations are strongly related to the structuration of tissues and membranes with the formation of lipoproteins.

Notes

1. Brenner, R. R. Presented for publication in Lipids.
2. Brenner, R. R. and A. Catala, manuscript in preparation.
3. Peluffo et al. Presented for publication.

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